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	$k_{inact} (s^{-1})$
CASPASE-1	$7.0 \times 10^{-6}$
CASPASE-3	$1.6 \times 10^{-5}$
CASPASE-7	$1.2 \times 10^{-6}$
CASPASE-8	$3.7 \times 10^{-5}$

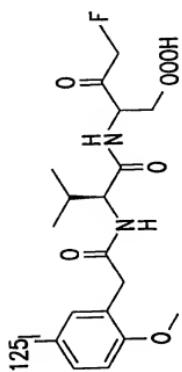
[<sup>125</sup>I]-M808

FIG. 1

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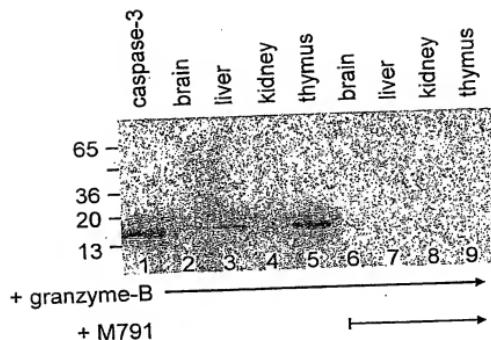


FIG. 2A

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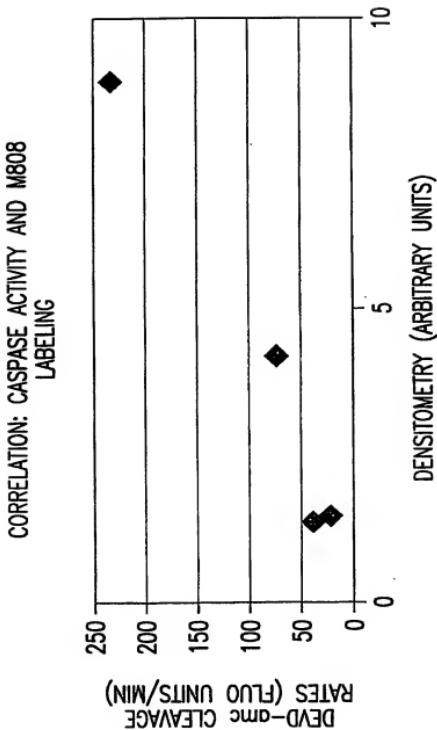


FIG. 2B

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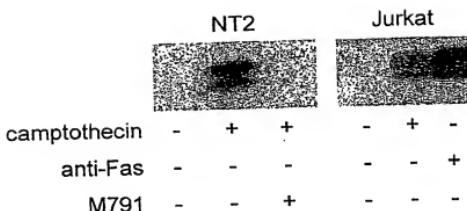


FIG.2C

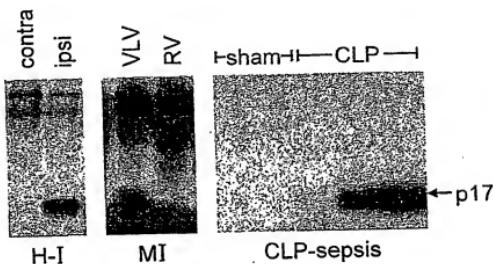


FIG.2D

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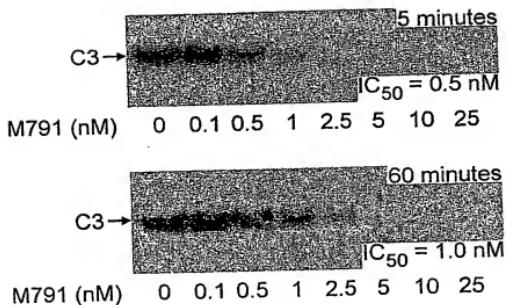


FIG.3A

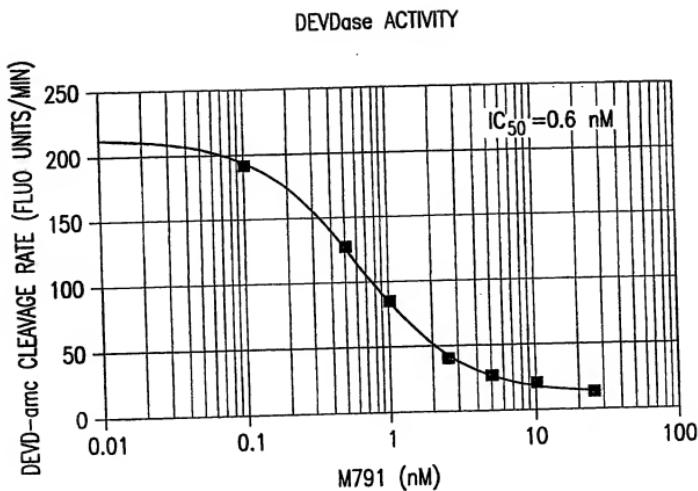


FIG.3B

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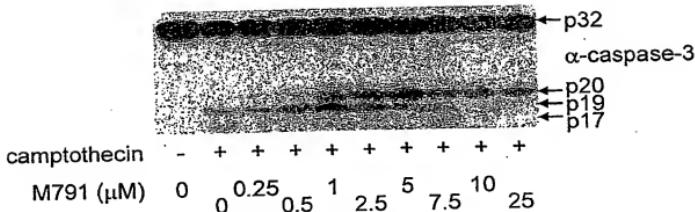


FIG.4A

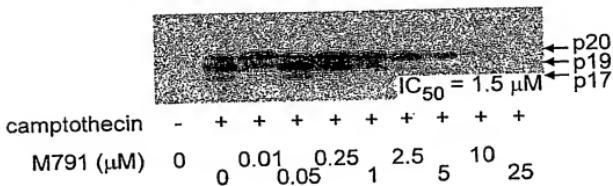


FIG.4B

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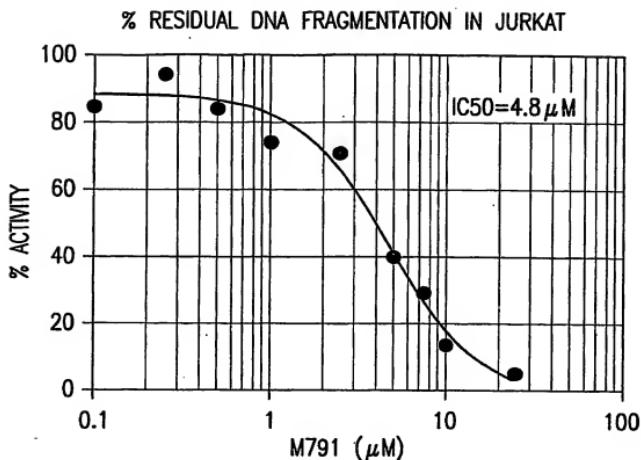


TABLE II. COMPARATIVE POTENCIES OF CASPASE INHIBITORS MEASURED BY M808 AND DNA FRAGMENTATION

CELL LINE	INHIBITOR	IC50 PROBE ( $\mu\text{M}$ )	IC50 DNA CLEAVAGE ( $\mu\text{M}$ )
NT2	M791	0.5	0.4
NT2	M826	0.045	0.03
NT2	M090	3.6	7
JURKAT	M791	2.5	4.8
JURKAT	M826	0.045	TO BE DETERMINED
THYMOCYTES	M826	0.07	0.14
THYMOCYTES	M867	0.09	0.27

FIG.4C

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- DETERMINE EXPECTED [ $^{125}$ I]-M808 SIGNAL IN DRUG-TREATED ANIMAL, BASED ON MEASURED TOTAL AMOUNT OF CASPASE-3, AS MEASURED BY WESTERN BLOTTING
- EXPECTED [ $^{125}$ I]-M808 SIGNAL: 3.6 UNITS
- ACTUAL [ $^{125}$ I]-M808 MEASURE: 1.0 UNIT
- FREE ENZYME =  $\frac{\text{ACTUAL}}{\text{EXPECTED}}$
- $= \frac{1.0 \text{ UNITS}}{3.6 \text{ UNITS}} = 0.28$

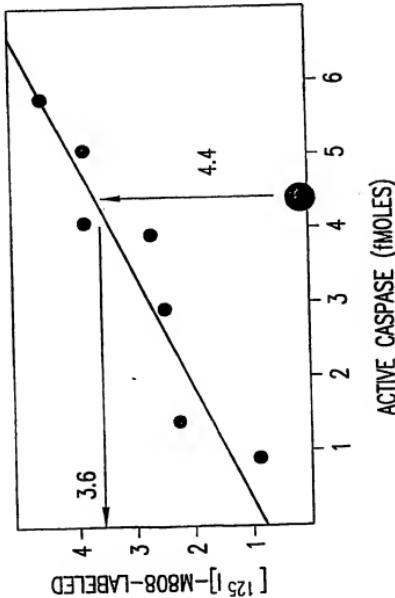


FIG. 5A

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Anti-caspase-3 western

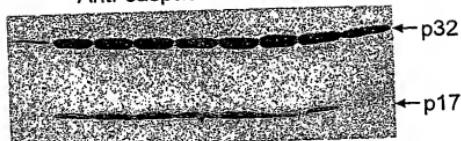


FIG. 5B

[<sup>125</sup>I]-M808 labeling

1 2 3 4 5 6 7 8 9

TABLE III. PERCENT OCCUPANCY OF CASPASE ACTIVE SITE BY M867 IN APOPTOTIC RAT THYMOCYTES									
LANE	1	2	3	4	5	6	7	8	9
M867 CONCENTRATION ( $\mu$ M)	0	0	0.005	0.025	0.05	0.1	0.25	1	5
p17 DENSITOMETRY	0.6	14.3	17.6	19.0	17.1	19.2	11.3	10.2	4.6
EXPECTED $[^{125}\text{I}]\text{-M808 SIGNAL}$ *			52.7	56.9	51.4	57.6	34.0	30.6	13.9
ACTUAL $[^{125}\text{I}]\text{-M808 SIGNAL}$	4.7	42.6	42.8	40.4	39.1	28.4	11.9	3.7	0.0
RATIO ACTUAL/EXPECTED			0.8	0.7	0.8	0.5	0.4	0.1	0.0
% FREE ACTIVE SITES			81	71	76	49	35	12	0
% OCCUPANCY			19	29	24	51	65	88	100

\* EXPECTED  $[^{125}\text{I}]\text{-M808 SIGNAL}$  CALCULATED BASED ON p17 DENSITOMETRY vs  $[^{125}\text{I}]\text{-M808 SIGNAL}$  IN ABSENCE OF DRUG

FIG. 5C

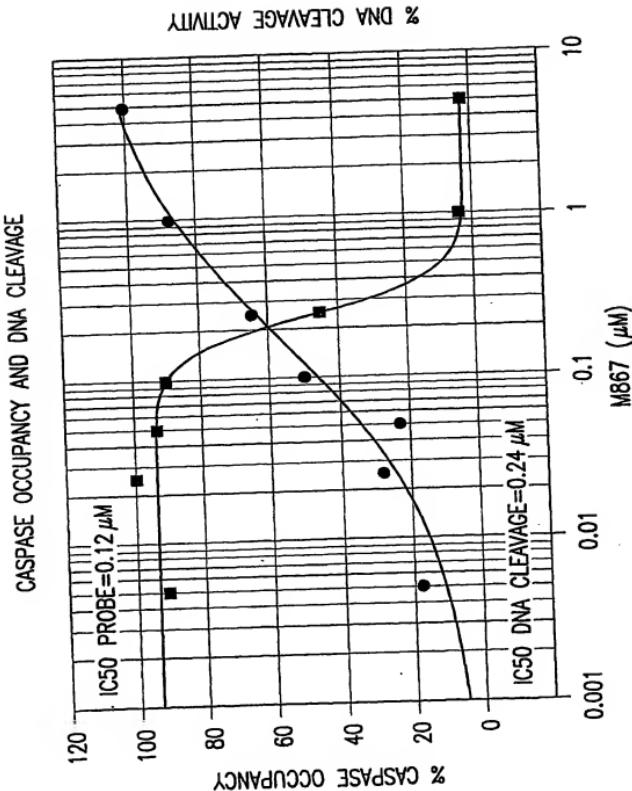


FIG. 5D

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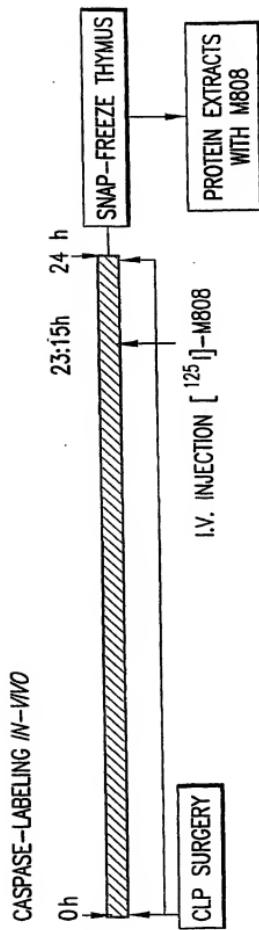


FIG. 6A

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FIG.6B

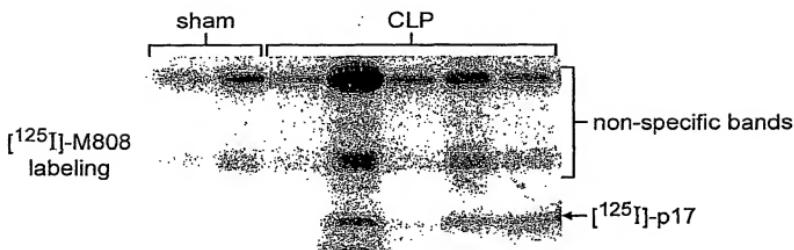


FIG.6C

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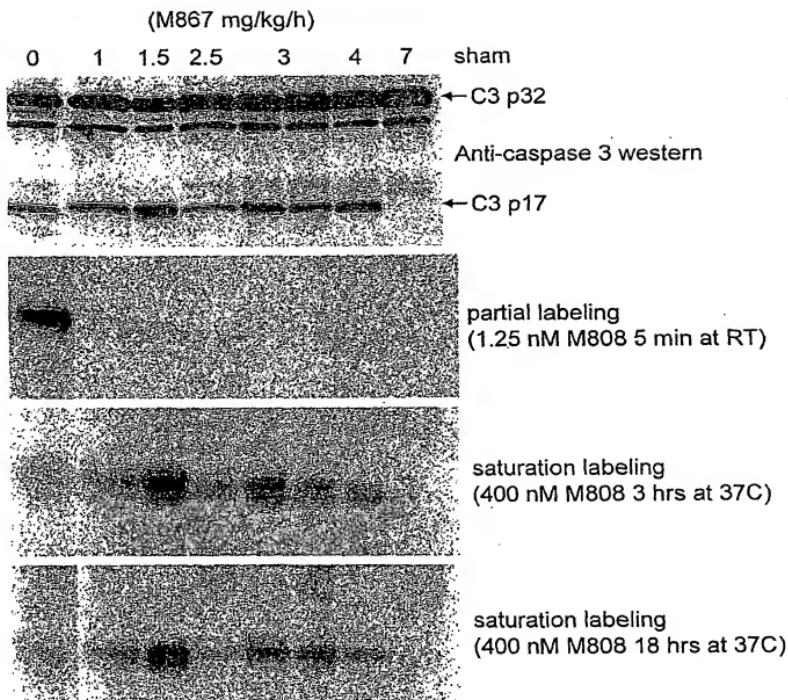


FIG.7

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